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TOXICITY OF PROPOSED WATER QUALITY CRITERIA-BASED MIXTURES OF 11 INORGANICS TO *CERIODAPHNIA DUBIA* AND FATHEAD MINNOW.

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EXECUTIVE SUMMARY

The State of South Dakota has proposed site-specific water quality criteria for 11 inorganics in streams receiving acid mine drainage from gold mining operations in the Black Hills. For nine inorganics, the proposed criteria exceed current national criteria. The chronic toxicity of a mixture of 11 inorganics combined at a ratio of their proposed Criterion Continuous Concentrations (pCCC) was assessed by an 8-day static-renewal test with ***Ceriodaphnia dubia.*** The acute toxicity of a mixture of the same inorganics combined at a ratio of their proposed Criterion Maximum Concentrations (pCMC) was assessed by a 96-h static-renewal test with fathead minnow. For the pCCC mixture, all of the C. ***dubia*** exposed to concentrations $\geq$1.0x pCCC died within 24 h and survival at concentrations 0.125x-0.50x pCCC was 30-60% after 8 days. Reproduction of C. ***dubia*** was significantly reduced at all mixture concentrations compared to the control treatment. These results indicate that the proposed site-specific CCC for these 11 inorganics are not protective of C. ***dubia*** if they occur simultaneously in the water. For the pCMC mixture, there were no mortalities of fathead minnow at concentrations $\leq$1.0x pCMC and the 96-h LC50 of the mixture was 1.52x pCMC. These results indicate that the proposed site-specific CMC for a mixture of these 11 inorganics is protective of fathead minnow. Additional studies are needed to determine protective concentrations of these inorganics combined in mixtures to C. ***dubia*** on an acute and chronic basis.

INTRODUCTION

The topography of the Northern Black Hills in South Dakota includes a variety of spring-fed cold water streams, which support several species of salmon and suckers along with diverse populations of aquatic invertebrates and aquatic plants. Four watersheds in the Northern Black Hills of South Dakota are currently impacted by acid mine drainage (AMD) produced by five large-scale active gold mines. Acid mine drainage from these mines is discharged into cold water streams after treatment. Inorganics commonly found in AMD from gold mining include arsenic, cadmium, chromium, copper, cyanide, lead, mercury, nickel, selenium, silver, and zinc (J. Gober, U.S. Fish and Wildlife Service, personal communication). The State of South Dakota, in cooperation with the mining companies, has proposed site-specific water quality criteria for these 11 inorganics in streams receiving AMD (Table 1). For nine of the eleven inorganics, the proposed site-specific criterion concentrations exceed current national water quality criteria derived by the U.S. Environmental Protection Agency (USEPA). The U.S. Fish and Wildlife Service is concerned that the proposed water quality criteria may not be protective of indigenous biota in these streams.

Current national water quality criteria for most inorganic pollutants consists of two numbers, the Criterion Continuous Concentration and the Criterion Maximum Concentration. The Criterion Continuous Concentration is the 4-day average concentration that should not be exceeded more than once every three years and is derived primarily from chronic toxicity data. The Criterion Maximum Concentration is the l-h average concentration that should not be exceeded more than once every three years and is derived from acute toxicity data (48-h and 96-h LC50s; Stephan *et al.* 1985). These criterion concentrations are based on data obtained from single-chemical toxicity and bioaccumulation tests with representative species. Consequently, these single-chemical water quality criteria do not account for mixtures of pollutants and may not be protective when these pollutants are present simultaneously in the environment. Moreover, it is not known if a mixture of the 11 inorganics found in AMD would adversely impact indigenous aquatic biota in streams of the Northern Black Hills if they were present at the proposed criterion concentrations.

The purpose of this study was to determine if mixtures of 11 inorganics combined at their proposed single-chemical criterion concentrations are toxic to ***Ceriodaphnia dubia*** and fathead minnow (*Pimephales* ***promelas).*** This approach does not assess the type and magnitude of interaction of the mixture components, but indicates whether these criteria are protective of aquatic biota when the inorganics are present as mixtures. ***Ceriodaphnia dubia*** and fathead minnow were chosen as test models because these species are routinely used in determining the acute and chronic toxicity of chemicals and effluents (Weber 1991, Weber ***et al.*** 1989).

METHODS AND MATERIALS

Test organisms

Ceriodaphnia dubia and fathead minnow were cultured in standard reconstituted hard water at 25 $\pm$ 1 °C following procedures similar to those given in Weber ***et al.*** (1989). The culture vessels were placed in temperature-controlled water baths under a photoperiod of 16 h light:8 h dark. The culture water had the following characteristics (mean $\pm$SD): hardness, 160 $\pm$ 1 mg/L as $CaCO_3$; alkalinity, 113 $\pm$3 mg/L as $CaCO_3$; pH, 8.19 $\pm$0.07; and conductivity, 554 $\pm$4 μmhos/cm @ 25°C.

Table 1. Proposed site-specific water quality criteria for 11 inorganics in selected streams of the Northern Black Hills of South Dakota.

	Criterion (µg/L)			
	Continuous Concentration		Maximum Concentration	
Inorganic	Proposed”	USEPA[b]	Proposed	USEPA
Arsenic (as As^{+3})	150	190	360	360
Cadmium”	3.4	1.6	6.7	6.7
Chromium (as Cr^{+6})	19	11	28	16
Copper”	47	18	59	28
Cyanide (as CN)	80	5.2	150	22
Lead”	19	5.8	484	149
Mercury	1.3	0.012	2.4	2.4
Nickel”	23	137	2,110	2,636
Selenium (as Se^{+4})	13	5.0[d]	41	20[d]
Silver”	4.7	-[e]	37	9.1
Zinc”	230	158	254	174

“Proposed site-specific water quality criteria for streams in the Northern Black Hills of South Dakota, J. Gober, U.S. Fish and Wildlife Service (personal communication).

[b]Current USEPA national water quality criteria (USEPA 1986).

‘Concentrations adjusted to a hardness of 160 mg/L as $CaCO_3$ using equations given by J. Gober, U.S. Fish and Wildlife Service (personal communication) and in USEPA (1986).

[d]Revised criterion (USEPA 1987).

“No Criterion Continuous Concentration available.

Ceriodaphnia were cultured individually in 30-mL glass beakers containing 15 mL of water. Individual cultures were started with neonates obtained from adults that had produced at least eight or more young in their third or subsequent broods and were less than 15 days old. *Ceriodaphnia* were fed 0.1 mL of a liquefied mixture of yeast, dried cereal leaves, and trout pellets (termed YCT) at a concentration of 1.65 mg solids/mL and 0.1 mL of *Selenastrum capricornutum* (termed algae) concentrate at a concentration of 3.0 x 10^7 cells/L.

Fathead minnows were obtained as l-day posthatch larvae from the Environmental and Contaminants Research Center, Columbia, MO. The larvae were shipped by air in a plastic bag inflated with oxygen and packed in a cooler. Upon arrival at the Yankton Ecotoxicology Research Station, the larvae were gradually acclimated to the test water over a 2-day period and to the test temperature over a 4-day period. The larvae were cultured under static-renewal conditions in a 18.9-L glass aquarium filled with 8 L of water. Each day, the culture water was renewed (c.a. 50-90% volume replacement) with fresh water and the larvae were fed 30 mL of a concentrated suspension of live brine shrimp nauplii (*Artemia* sp.) three times per day.

Test water

Both tests were conducted in standard reconstituted hard water prepared by adding appropriate amounts of reagent-grade calcium sulfate dihydrate, magnesium sulfate, potassium chloride, and sodium bicarbonate to deionized (DI) water in a polyethylene carboy or tank and then vigorously aerating the water for at least 1 day (Weber *et al.* 1989). Each batch of water prepared was analyzed following standard procedures (APHA *et al.* 1989) to insure that the water quality met the criteria for hardness, alkalinity, and pH. The untreated test water had the following characteristics (mean ±SD): hardness, 162 ±2 mg/L as $CaCO_3$; alkalinity, 111 ±1 mg/L as $CaCO_3$; pH, 8.19 ±0.09; and conductivity, 551 ±3 μmhos/cm @ 25°C.

Test design

The approach used in this study was to treat each mixture as one entity. For one mixture, the 11 inorganics were combined at a fixed ratio of their proposed Criterion Continuous Concentrations (pCCC) and for the other mixture the same 11 inorganics were combined at a fixed ratio of their proposed Criterion Maximum Concentrations (pCMC; Table 1). For simplicity, the mixtures are designated as the pCCC mixture and pCMC mixture and the concentrations of the mixtures are expressed as multiples of the pCCC and pCMC. The seven mixture concentrations used in both tests were 0.125x, 0.25x, 0.50x, 1.0x, 2.0x, 4.0x, and 8.0x the criterion concentrations.

Test chemicals

The chemicals used in the tests and their sources are given in Table 2. The percentage of inorganic toxicant in each chemical was determined from the certificate of analysis provided by the supplier. Individual stock solutions of the 11 inorganics were prepared in DI water on the day before the start of each test and stock solutions at concentrations < 100 mg/L were prepared daily during each test. The high mixture concentration (8.0x) was prepared first by diluting appropriate aliquots of each stock solution with reconstituted hard water in a volumetric flask. The remaining test solutions were prepared by diluting appropriate volumes of the high concentration with reconstituted hard water in volumetric flasks, except for lead

Table 2. Compositional percentages and suppliers of inorganic compounds used in mixture tests with *Ceriodaphnia dubia* and fathead minnow.

Compound	Formula	Composition	CAS" Registry No.	Supplier[b]
Sodium arsenite	$NaAsO_2$	56.8% As^{+3}	7784-46-5	1
Cadmium chloride	$CdCl_2$	61.1% Cd	10108-64-2	2
Sodium chromate tetrahydrate	$Na_2CrO_4 \cdot 4H_2O$	22.5 % Cr^{+6}	10034-82-9	2
Copper sulfate pentahydrate	$CuSO_4 \cdot 5H_20$	25.2% cu	7758-99-8	2
Sodium cyanide	NaCN	51.9% CN	143-33-9	2
Lead nitrate	$Pb(NO_3)_2$	61.2% Pb	10099-74-8	2
Mercury II chloride	$HgCl_2$	72.5% Hg	7487-94-7	2
Nickel (II) sulfate hexahydrate	$NiSO_4 \cdot 6H_2O$	22.2% Ni	10101-97-0	1
Silver nitrate	$AgNO_3$	63.5% Ag	7761-88-8	3
Sodium selenite	Na_2SeO_3	46.6% Se^{+4}	10102-18-8	2
Zinc sulfate heptahydrate	$ZnSO_4 \cdot 7H_2O$	22.5% Zn	7446-20-o	2

[a]CAS, Chemical Abstract Service.

[b]Supplier: 1, J.T. Baker Chemical Co.; 2, Aldrich Chemical Co.; 3, Fisher Scientific.

nitrate in the pCMC mixture test. For the pCMC mixture test, appropriate aliquants of lead nitrate stock solution were added seperately to each treatment preparation flask because the addition of lead nitrate to the high treatment formed a precipitate. For the control treatment, an aliquot of DI water equivalent to the volume of the 11 stock solutions used to prepare 1 L of the high concentration was brought to volume with reconstituted hard water in a 1000-mL volumetric flask. All test concentrations are based on nominal concentrations of the inorganic toxicant added.

The proposed site-specific criteria did not specify the oxidation state for arsenic, chromium, and selenium. Arsenite (As^{+3}) was used because national water quality criteria are available for this form, but not for arsenate (As^{+5}; USEPA 1986). Hexavalent chromium (Cr^{+6}) was used because the proposed site-specific criteria for total chromium and national water quality criteria for hexavalent chromium are not adjusted for water hardness, whereas national water quality criteria for trivalent chromium (Cr^{+3}) are adjusted for water hardness. Selenite (Se^{+4}) was used because it is generally more toxic to aquatic biota than selenate (Se^{+6}; USEPA 1987) and earlier national criteria were based on selenite (USEPA 1986).

Chronic toxicity testing

The three brood *Ceriodaphnia dubia* survival and reproduction test described in Weber *et al.* (1989) was used to estimate the chronic toxicity of the pCCC mixture. *Ceriodaphnia* were tested in 30-mL beakers containing 15 mL of solution at 25 ± 1 “C. Each mixture concentration and control treatment had 10 replicates and one animal was stocked in each replicate. The test was initiated with < 24-h-old neonates (within 8 h of the same age) nitrate in the pCMC mixture test. For the pCMC mixture test, appropriate aliquants of lead nitrate stock solution were added seperately to each treatment preparation flask because the addition of lead nitrate to the high treatment formed a precipitate. For the control treatment, an aliquot of DI water equivalent to the volume of the 11 stock solutions used to prepare 1 L of the high concentration was brought to volume with reconstituted hard water in a 1000-mL volumetric flask. All test concentrations are based on nominal concentrations of the inorganic toxicant added.

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Each day, all live F_0 *Ceriodaphnia* were transferred (using a glass pipet) to new test solutions in a second set of 30-mL beakers and fed 0.1 mL of YCT solution and 0.1 mL of algae concentrate. The number of living and dead young produced each day were counted and discarded. The test was terminated after 8 days when at least 60% of the control animals released three broods of neonates.

Dissolved oxygen (YSI model 58 dissolved oxygen meter), pH (Orion models 250A pH meter and 9107 pH electrode), and temperature (same as pH) of the old and new test solutions were measured daily in each treatment with live animals. Because of the small volume of solution in the test beakers, dissolved oxygen, pH, and temperature were measured on a composite sample of the old test solution and on a 100-mL sample of the new test solution. Water from the control, low, medium, and high treatments (with live animals) was collected from the exposure beakers on Days 1 and 7 of exposure and from the test solution preparation flasks on Days 0 and 6 of exposure for analysis of hardness, alkalinity, pH, and conductivity.

Acute toxicity testing

The fathead minnow static-renewal 96-h test described in Weber (1991) was used to estimate the acute toxicity of the pCMC mixture. Fathead minnow larvae were tested in

400-mL beakers containing 300 mL of solution at 25 $\pm$ 1 °C, with 2 replicates per treatment. The test was initiated with 7-day posthatch larvae and groups of 10 fish were randomly stocked into each duplicate test beaker. The beakers were allocated to random positions in a temperature-controlled water bath under a photoperiod of 16 h light:8 h dark and covered with glass plates. Light intensities measured at 10 cm above the water surface at test initiation ranged from 90 to 94 footcandles.

Observations on mortality and overt behavioral alterations were made at 24-h intervals and all dead fish were removed. Fish without perceivable movement when gently prodded were removed from the beaker and examined under 30x magnification for the absence of a heartbeat, which was the criterion for death. After each observation, the test solution was renewed by siphoning out about 225 mL of old test solution and replacing it with an equivalent volume of newly prepared test solution. At 46 h of exposure (2 h before the renewals), the fish were fed 0.1 mL of a concentrated solution of live brine shrimp nauplii. Prior to use, the brine shrimp were filtered through a 153 μm screen and resuspended in reconstituted hard water. Mean ($\pm$ SD) total length of the control fish at the end of test was 8.3 (f0.6) mm and their mean weight determined from a pooled sample of 19 fish was 4 mg.

Dissolved oxygen, pH, and temperature of the old and new test solutions were measured daily (as described above) in each beaker with live fish. Water from the control, low, medium, and high treatments (with live fish) was collected from the test solution preparation flasks at 0 and 72 h of exposure and from each beaker at 96 h of exposure for analysis of hardness, alkalinity, pH, and conductivity.

Analytical

Two sets of water samples were collected from each test solution preparation flask at Days 0 and 6 (for treatments with live animals) of the pCCC mixture test and at 0 h of the pCMC mixture test. One set of samples for analysis of arsenic, mercury, and selenium was preserved in 1% HCl and stored at -20°C and the other set of samples for analysis of cadmium, chromium, copper, lead, nickel, silver, and zinc was preserved in 1% HNO_3 and stored at -20°C. These samples will be used to document exposure concentrations of the inorganics (except cyanide) if questions arise.

Data analysis

Data from the pCCC test were analyzed by standard statistical procedures given in Weber ***et al.*** (1989). Survival data were analyzed by Fisher's Exact test to identify differences (p = 0.05) between the control and treatment groups to determine the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) for survival. For the reproduction data, the assumptions of normality and homogeneity of variance were tested by Shapiro-Wilk's test and Bartlett's test, respectively. Because these assumptions were met, data for total number of young produced per adult were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's T-test to identify differences (p = 0.05) between the control and treatment groups to determine the NOEC and LOEC for reproduction. The reproduction data were also subjected to linear interpolation analysis to calculate the inhibition concentration (IC) for a 25% and 50% reduction in the number of young produced.

The LC50 values and their 95% confidence intervals were estimated by the

Spearman-Karber method (Weber 1991). Mortality data from replicate treatments of the pCMC mixture test were combined before the LC50s were calculated. Routine statistical analysis of water quality data were performed using SAS programs (SAS 1990).

RESULTS

The chemical characteristics of the test waters for both studies are given in Table 3. Hardness, alkalinity, and conductivity of the test water were similar (*p* = 0.05) in both studies. Hardness could not be determined in the medium and high treatments of either mixture test because of matrix interferences with the colorimetric assay. Dissolved oxygen concentrations were maintained at ≥6.7 mg/L (> 80% saturation) in all test solutions. Differences in pH between the controls and mixture solutions were within ≤0.1 unit for the pCCC mixture test and ≤0.2 unit for the pCMC test.

pCCC ***mixture***

Mixture concentrations equal to or greater than the pCCC killed all of the C. ***dubia*** within 24 h of exposure (Table 4). An inverted concentration-response relation for survival was obtained for C. ***dubia*** exposed to mixture concentrations of 0.125x to 0.50x pCCC. After 8 days of exposure, survival at 0.50x pCCC was 60%; whereas at 0.125x and 0.25x pCCC it was 50% and 30%, respectively. Although survival at these mixture concentrations was 33 to 67% lower than in the control treatment, only the reduction in survival at 0.25x pCCC was statistically significant (*p* = 0.05). The estimated 48-h LC50 (95% confidence interval) of the pCCC mixture was 0.66x (0.57-0.76).

Table 3. Chemical characteristics of exposure water for the proposed Criterion Continuous Concentrations (pCCC) and proposed Criterion Maximum Concentrations (pCMC) mixture tests with ***Ceriodaphnia dubia*** and fathead minnow.

Characteristic (unit)	Mixture test[a]	
	p c c c	pCMC
Hardness (mg/L as $CaCO_3$)	163 ±2 (160-167)	164 ±2 (162-168)
Alkalinity (mg/L as $CaCO_3$)	112 ±1 (111-114)	113 ±1 (111-115)
Conductivity (μmhos/cm @ 25°C)	558 ±7 (546-570)	562 ±18 (538-599)
Dissolved oxygen (mg/L)	7.3 f0.2 (6.9-7.8)	7.6 ±0.5 (6.7-8.6)
pH (pH units)	8.27 ±0.06 (8.11-8.38)	8.14 ±0.05 (8.00-8.24)
Temperature (“C)	24.7 f0.2 (24.4-25 .O)	24.8 f0.2 (24.5-25.2)

“Values are mean ± SD, with the range in parentheses.

Reproduction of C. *dubia* at the three lowest mixture concentrations (0.125x-0.50x pCCC) was significantly reduced ($p = 0.05$) compared to the control treatment after 8 days of exposure (Table 4). The number of young produced per female was inversely correlated with mixture concentration (r = -0.772, p = O.OOOl), which indicated that the magnitude of this effect showed a significant concentration-response relation at these concentrations.

A maximum acceptable toxicant concentration (MATC; geometric mean of the NOEC and LOEC) could not be determined for this mixture because reproduction of C. *dubia* was significantly reduced at all mixture concentrations. Alternately, the ICs (95% confidence interval) for a 25% and 50% reduction in young produced were 0.10x (0.08-0.19) and 0.33x (0.20-0.50) pCCC, respectively.

pCMC mixture

There were no mortalities among fathead minnow exposed to mixture concentrations at or below the pCMC, whereas mixture concentrations at 2 to 8 times the pCMC for the 11 inorganics killed ≥90 % of the fish within 48 h (Table 5). Most of the mortality occurred within the first 24 h of exposure and acute lethality ceased within 48 h of exposure. The estimated 24-h LC50 of 1.57x pCMC was very close to the 96-h LC50 of 1.52x pCMC.

DISCUSSION

The results of this study clearly show that the proposed site-specific CCC for these 11 inorganics are not protective of C. *dubia* under the conditions tested, if all 11 inorganics are present at concentrations ≥0.125x pCCC. Moreover, the occurrence of these 11 inorganics in

Table 4. Survival and reproduction of ***Ceriodaphnia dubia*** exposed to a mixture of 11 inorganics combined at a ratio of their proposed Criterion Continuous Concentrations (pCCC) for 8 days in reconstituted hard water.

	Biological endpoint”	
Treatment	Survival (%)	Number of young/female[b]
Control	90	20.6 ±4.2
0.125x pccc	50	14.4 ±2.8*
0.25x pccc	30*	11.3 ±4.6*
0.50x pccc	60	8.2 ±3.7*
1.0x pccc	0”	0
2.0x pccc	0*	0
4.0x pccc	0*	0
8.0x pCCC	0*	0

“Asterisks denote values that are significantly different ($p = 0.05$) from the control treatment for a given endpoint.

[b]Values are mean ± SD.

Table 5. Survival of fathead minnow exposed to a mixture of 11 inorganics combined at a ratio of their proposed Criterion Maximum Concentrations (pCMC) for 96 h in reconstituted hard water.

Treatment	Survival (%) at 24 h	48 h	96 h
Control	100	95	95
0.125x pCMC	100	100	100
0.25x pCMC	100	100	100
0.50x pCMC	100	100	100
1.0x pCMC	100	100	100
2.0x pCMC	15	10	10
4.0x pCMC	0	0	0
8.0x pCMC	0	0	
LC50 (as x pCMC) and 95 % confidence interval in parentheses.	1.57 (1.40-1.76)	1.52 (1.38-l .67)	1.52 (1.38-1.67)

streams at concentrations equal to or greater than their pCCC may completely eliminate populations of C. ***dubia*** and other aquatic biota with similar or greater sensitivities, as evidenced by complete mortality of C. ***dubia*** within 24 h of exposure to these mixture concentrations.

A mixture of the same 11 inorganics combined at multiples of their pCMC did not adversely affect the survival of fathead minnow at concentrations less than or equal to pCMC over a 4-day period. However, brief excursions of concentrations at or above 1.5x the pCMC may be acutely lethal to fathead minnows.

Comparisons between the mixtures are limited to acute effects because the pCMC mixture was tested only for lethality over a 4-day period. Based on 48-h LC50 values, the pCCC mixture was more toxic to C. ***dubia*** (0.66x pCCC) than was the pCMC mixture to fathead minnow (1.52x pCMC). The concentrations of each inorganic at the 48-h LC50 of the pCMC mixture were 3 to 211 times higher than those at the 48-h LC50 of the pCCC mixture. These results indicate that C. ***dubia*** are more sensitive than fathead minnow to the inorganics tested as mixtures. Similarly, Spehar and Fiandt (1986) tested a mixture of six inorganics (As^{+3}, Cd, Cr^{+6}, Cu, Hg, and Pb) combined at a ratio of their national CMC proposed in 1984 and found that C. ***dubia*** were more acutely sensitive to the mixture than fathead minnow. They also reported that C. ***dubia*** were more sensitive to five of the six inorganics tested individually than fathead minnow, even though C. ***dubia*** were tested in water with a higher hardness (100 mg/L as $CaCO_3$) compared to that used for the fathead minnow tests (44 mg/L as $CaCO_3$).

Inorganics combined in mixtures may interact to produce joint effects on aquatic biota.

The types of joint toxicity exhibited by inorganic mixtures are commonly classified as additive, synergistic, or antagonistic depending on the relation of the toxicity of the mixture to that of the individual components. Techniques for quantitatively evaluating the joint toxicity of chemical mixtures are available, but require testing the mixture and each component separately (Marking 1977, Konemann 1981). In contrast to joint action, chemicals combined in mixtures may also show no interaction (Sprague 1970).

Without testing each inorganic individually, it is not possible to quantitatively assess the joint toxicity of these mixtures using the additive index of Marking (1977) or the mixture toxicity index of Konemann (1981). However, the toxic unit (TU) concept discussed by Sprague (1970) was used to provide an initial assessment of the joint toxicity and the major toxic components of each mixture. The theoretical toxic contribution of each component to the toxicity of the mixture is expressed as a TU and the TUs are summed to estimate the type of interaction among the components in the mixture. A TU is defined as the concentration of a chemical in a toxic mixture divided by its individual toxic concentration for the endpoint measured (i.e., its individual IC50 or 96-h LC50 value). Mixtures with summed TU values close to 1 .O are considered to be additive in toxicity, those with summed TU $>$ 1 .O are less than additive in toxicity (antagonistic), and those with summed TU $<$ 1 .O are greater than additive in toxicity (synergistic). Because the inorganic components in the mixtures were not tested individually, the theoretical TUs were estimated by using individual toxicity values reported in the literature for C. *dubia* (when found) and fathead minnow. In cases where toxicity values were not found for C. *dubia,* chronic values for the species most closely related taxonomically to C. *dubia* were used. The TUs derived in Tables 6 and 7 should be considered as only approximations, because the toxicity values are not directly comparable due to differences in test conditions (e.g., species, age, size, and quality of test organisms, water quality, test chambers, experience, etc.) used in this study and those cited. For those inorganics in which their criterion values were adjusted for water hardness, an attempt was made to limit the comparisons to studies that used water with a hardness closest to 160 mg/L as $CaCO_3$. For silver and lead, the individual 96-h LC50 values used to estimate the theoretical TUs for the pCMC mixture were calculated for a hardness of 160 mg/L as $CaCO_3$ from regression equations of the natural logarithms of 96-h LC50 values on natural logarithms of hardness values for tests with fathead minnow given in USEPA water quality criteria documents (USEPA 1980,1984).

Summations of the theoretical TUs indicate that the joint toxicity of both mixtures was less than additive and that the magnitude of the less than additive action for both mixtures was similar (summed TUs, 3.06 for the pCCC mixture and 3.76 for the pCMC mixture; Tables 6 and 7). To assess the potential major toxic components, the theoretical TU of each inorganic was expressed as a percentage of the summed TUs. Based on this analysis, cyanide was the major toxic component in both mixtures, it theoretically contributed about 42% and 50% of the toxicity (as summed TUs) of the pCCC mixture and pCMC mixture, respectively. In contrast, arsenic, lead, mercury, and selenium each contributed ≤ 1% , chromium contributed ≤2 % , and nickel contributed about 3 % of the toxicity of both mixtures. The theoretical toxic contributions of the other inorganics varied between mixtures. In the pCCC mixture, the theoretical toxic contribution of zinc (24%) was more than twice that of silver (10%); whereas in the pCMC mixture, the theoretical toxic contribution of silver (25 %) was more than twice

Table 6. Comparisons of inorganic concentrations at the IC50[a] of the proposed Criterion Continuous Concentrations mixture for *Ceriodaphnia dubia* (0.33x pCCC) to published individual chronic toxicity values for freshwater invertebrates.

Inorganic	Concentration at the IC50 (μg/L)	Individual chronic toxicity value and test conditions: chronic value (μg/L)	Endpoint[b]	Species	Hardness[c]	Reference[d]	Toxic unit[e]	Percent of summed toxic units
Arsenic (as As^{+3})	*49.5*	1,259	EC50	*Ceriodaphnia dubia*	100	1	*0.04*	1.3%
Cadmium	*1.12*	6.0	EC50	*Ceriodaphnia dubia*	100	1	*0.19*	6.2%
Chromium (as Cr^{+6})	*6.27*	132	EC50	*Ceriodaphnia dubia*	100	1	*0.05*	1.6%
Copper	*15.5*	56	EC50	*Ceriodaphnia dubia*	100	1	*0.28*	9.2%
Cyanide (as HCN)	23.8[f]	18.3	MATC	*Gammarus pseudolimnaeus*	NR[g]	2	*1.30*	42.5%
Lead	*6.27*	269	EC50	*Ceriodaphnia dubia*	100	1	*0.02*	0.7%
Mercury	*0.429*	12.6	EC50	*Ceriodaphnia dubia*	100	1	*0.03*	1.0%
Nickel	*7.59*	95	EC50	*Daphnia magna*	45	3	*0.08*	2.6%

Table 6. Continued.

Inorganic	Concentration at the IC50 (μg/L)	Individual chronic toxicity value and test conditions: chronic value (μg/L)	Endpoint[b]	Species	Hardness[c]	Reference[d]	Toxic unit[e]	Percent of summed toxic units
Selenium (as Se^{+4})	4.29	280	MATC	*Ceriodaphnia affinis*	101	4	0.02	0.7%
Silver	1.55	5.2	MATC	*Daphnia magna*	180	5	0.30	9.8%
Zinc	76	102	EC50	*Daphnia magna*	45	3	0.75	24.5%

[a]IC50, Inhibition concentration causing 50% reduction in number of young produced relative to the control.

[b]EC50, Concentration causing 50% reduction in number of young produced; MATC, Maximum acceptable toxicant concentration based on reproduction, calculated as the geometric mean of the no observed effect concentration and lowest observed effect concentration.

[c]mg/L as $CaCO_3$.

[d]Reference: 1, Spehar and Fiandt 1986; 2, Smith *et al.* 1979; 3, Biesinger and Christensen 1972; 4, Owsley and McCauley 1986; 5, Nebeker *et al.* 1983.

Concentration at the IC50 of the pCCC mixture ÷ its individual chronic toxicity value. Summed toxic units = 3.06.

[f]Concentration of HCN was calculated for the free cyanide (HCN and CN^-) concentration of 26.4 μg/L using the mean pH and temperature of the pCCC mixture test solutions and the equilibrium equations given in Broderius and Smith (1979).

[g]Not reported.

Table 7. Comparisons of inorganic concentrations at the 96-h LC50 of the proposed Criterion Maximum Concentrations mixture for fathead minnow (1.52x pCMC) to published individual acute toxicity values for fathead minnow.

	Concentration at 96-h LC50	Individual acute toxicity value and test conditions					
Inorganic	of mixture (μg/L)	96-h LC50 (μg/L)	Age, size, or life stage	Hardness[a]	Reference[b]	Toxic unit[c]	Percent of summed toxic units
Arsenic (as As^{+3})	547	12,600	30-d old (0.15g)	44	1	0.04	1.1%
Cadmium	10.2	90	14-30-d old	200	2	0.11	2.9%
Chromium (as Cr^{+6})	42.6	43,300	30-d old (0.15g)	44	1	< 0.01	<0.1%
Copper	89.7	490	6-week old	202	3	0.18	4.8%
Cyanide (as HCN)	211[d]	113	Swim-up fry (5-6 mm)	220	4	1.87	49.7%
Lead	736	125,500”	NR[f]	160	5	0.01	0.3%
Mercury	3.65	168	Juvenile (3-month old)	46	6	0.02	0.5%
Nickel	3,207	27,900[g]	Immatures	207	7	0.11	2.9%

Table 7. Continued.

Inorganic	Concentration at 96-h LC50 of mixture (µg/L)	Individual acute toxicity value and test conditions: 96-h LC50 (µg/L)	Size, age, or life stage	Hardness[a]	Reference[b]	Toxic unit”	Percent of summed toxic units
Selenium (as Se^{+4})	62.3	1,700	30-d old	51	8	0.04	1.1%
Silver	56.2	60”	NR	160	9	0.94	25.0%
Zinc	386	870	l-d old	174-198	10	0.44	11.7%

[a]mg/L as $CaCO_3$.

[b]Reference: 1, Spehar and Fiandt 1986; 2, Hall *et al.* 1986; 3, Pickering *et al.* 1977; 4, Smith *et al.* 1978; 5, USEPA 1984; 6, Snarski and Olson 1982; 7, Pickering 1974; 8, USEPA 1987; 9, USEPA 1980; 10, Pickering and Vigor 1965.

‘Concentration at the 96-h LC50 of the pCMC mixture ÷ its individual 96-h LC50 value. Summed toxic units = 3.76.

[d]Concentration of HCN was calculated for the free cyanide (HCN and CN^-) concentration of 228 µg/L using the mean pH and temperature of the pCMC mixture test solutions and the equilibrium equations given in Broderius and Smith (1979).

“96-h LC50 value estimated from least squares regression of the natural logarithms of 96-h LC50 values on natural logarithms of hardness values for tests with fathead minnow given in the reference.

[f]Not reported.

[g]Geometric mean of four values.

that of zinc (12%). The theoretical toxic contributions of cadmium and copper were about twofold higher in the pCCC mixture compared to the pCMC mixture.

These preliminary findings indicate that the toxicity of the proposed water quality-based mixtures was probably due to the mixture of cadmium, copper, cyanide, silver, and zinc, which accounted for 92-94% of the summed toxic units. The toxic contribution of the remaining inorganics to these mixtures was probably minor.

CONCLUSIONS

The results of this study indicate that the proposed site-specific CCC of these 11 inorganics for streams in the Northern Black Hills are not protective of C. *dubia* if they are present as a mixture. Although the mixture of 11 inorganics combined at the pCMC was not acutely toxic to fathead minnow, this mixture should be tested with C. *dubia* because of their high sensitivity to the pCCC mixture. Additional studies are needed to determine the type of joint action and major toxic components of these mixtures. This information may be used to adjust the site-specific criterion values for certain inorganics to reduce the toxicity of the whole 11 -inorganic mixture.

REFERENCES

APHA (American Public Health Association, American Water Works Association, and Water Pollution Control Federation). 1989. *Standard Methods for the Examination of Water and Wastewater,* 17th ed. American Public Health Association, Washington, DC.

Biesinger, K.E. and G.M. Christensen. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna.* J. Fish. Res. Board Can. 29: 1691-1700.

Broderius, S. J. and L.L. Smith, Jr. 1979. Lethal and sublethal effects of binary mixtures of cyanide and hexavalent chromium, zinc, or ammonia to the fathead minnow (*Pimephales promelas)* and rainbow trout (*Salmo gairdneri).* J. Fish. Res. Board Can. 36: 164-172.

Hall, W.S., R.L. Paulson, L.W. Hall, Jr, and D.T. Burton. 1986. Acute toxicity of cadmium and sodium pentachlorophenate to daphnids and fish. Bull. Environ. Contam. Toxicol. 37:308-316.

Konemann, H. 1981. Fish toxicity tests with mixtures of more than two chemicals: A proposal for a quantitative approach and experimental results. Toxicology 19:229-238.

Marking, L.L. 1977. Method for assessing additive toxicity of chemical mixtures. Pages 99-108 *in* F.L. Mayer and J.L. Hamelink, eds. Aquatic Toxicology and Hazard Evaluation, ASTM STP 634. American Society for Testing and Materials, Philadelphia, PA.

Nebeker, A.V., C.K. McAuliffe, R. Mshar, and D.G. Stevens. 1983. Toxicity of silver to steelhead and rainbow trout, fathead minnows and *Daphnia magna.* Environ. Toxicol. Chem. 2:95-104.

Owsley, J.A. and D.E. McCauley. 1986. Effect of extended sublethal exposure to sodium selenite on *Ceriodaphnia affinis.* Bull. Environ. Contam. Toxicol. 36:876-880.

Pickering, Q.H. 1974. Chronic toxicity of nickel to the fathead minnow. J. Water Pollut. Control Fed. 46:760-765.

Pickering, Q.H. and W .N. Vigor. 1965. The acute toxicity of zinc to eggs and fry of the fathead minnow. Prog. Fish Cult. 27:153-157.

Pickering, Q., W. Brungs, and M. Gast. 1977. Effect of exposure time and copper concentration on reproduction of the fathead minnow (*Pimephales promelas).* Water Res. 11:1079-1083.

SAS Institute. 1990. ***SAS Procedures Guide,*** Version 6, 3rd ed. SAS Institute, Cary, NC. ***705*** pp.

Smith, L.L., Jr., S.J. Broderius, D.M. Oseid, G.L. Kimball, and W.M. Koenst. 1978. Acute toxicity of hydrogen cyanide to freshwater fishes. Arch. Environ. Contam. Toxicol. 7:325-337.

Smith, L.L., Jr., S.J. Broderius, D.M. Oseid, G.L. Kimball, W.M. Koenst, and D.T. Lind. 1979. Acute and chronic toxicity of HCN to fish and invertebrates. EPA-600/3-79-009. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN. 115 pp.

Snarski, V.M. and Olson, G.F. 1982. Chronic toxicity and bioaccumulation of mercuric chloride in the fathead minnow (*Pimephales promelas*). Aquat. Toxicol. 2: 143-156.

Spehar, R.L. and J.T. Fiandt. 1986. Acute and chronic effects of water quality criteria-based metal mixtures on three aquatic species. Environ. Toxicol. Chem. 5:917-931.

Sprague, J.B. 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Res. 4:3-32.

Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN. 98 pp.

USEPA. 1980. Ambient water quality criteria for silver. EPA 440/5-80-071. U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC.

USEPA. 1984. Ambient water quality criteria for lead-1984. EPA 440/5-84-027. U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC.

USEPA. 1986. Quality criteria for water 1986. EPA 440/5-86-001. U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington DC.

USEPA. 1987. Ambient water quality criteria for selenium- 1987. EPA-440/5-87-006. U. S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC. 121 pp.

Weber, C.I. 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 4th ed. EPA/600/4-90/027. U.S. Environmental

Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, OH. 293 pp.

Weber, C.I. and 13 others. 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 2nd ed. EPA/600/4-89/001. U. S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, OH. 249 pp.